Breeding Objectives in Forages

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1 Introduction

All breeding programs share one common objective – to improve a species for use within a target population of environments and a particular agricultural context. Beyond this common goal, the objectives of forage breeding programs are as varied as the species upon which they are based and the breeders who develop and implement them. Many breeding objectives are determined a priori by the choice of a species with one or more obvious trait limitations or deficiencies, such as poor seedling vigor, synthesis of toxic alkaloids, or severe susceptibility to a major pest. For species without such limitations, breeders have the luxury of defining less stringent and/or more flexible breeding objectives. This may allow breeders to focus their efforts on improving breeding methods and efficiency, develop additional breeding objectives or selection criteria, or simply to increase the size or scope of the breeding program.

Breeding objectives are framed within the agricultural context and the environments in which the species will be used (Figure 1). Management and/or environmental constraints that impose stress upon a species should be used to develop breeding objectives and methodologies that allow selection for tolerance or resistance to the stresses imposed. In some cases, this can be accomplished by dissecting complex traits into simpler traits, such as using a simple freezing tolerance test to select plants with improved freezing tolerance, improved persistence in the field, and increased long-term yield stability. In other cases, certain managements or environments may obviate the need to focus on particular breeding objectives that are otherwise important. For example, toxic alkaloids of reed canarygrass (*Phalaris arundinacea* L.) are metabolized to non-toxic compounds in dry hay, eliminating the need to breed for reduced alkaloid concentrations in reed canarygrass cultivars to be used for hay production.

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Fig. 1 Illustration of the trilateral relationship of target species, agricultural context, and target population of environments in determining forage breeding objectives

2 Growth Characteristics

2.1 Evaluation of Phenotype: Spaced Plants Versus Swards

The vast majority of forage breeding is conducted in spaced-plant nurseries, which are comprised of hundreds or thousands of plants on a regular spacing, designed for efficient collection of individual plant samples and/or data. Nearly all spaced-plant nurseries use spacings sufficiently wide to allow easy movement among plants for sample and data collection and to maintain the integrity of each genotype, eliminating competition between neighboring plants and creating a uniform environment for maximum phenotypic expression of each plant. As a result, spaced-plant nurseries bear no resemblance to the real world of forage production in swards, particularly mixed swards of multiple species, in which there are high rates of seedling and adult-plant mortality, interplant competition, and potential seedling recruitment, each of which can cause dynamic changes to sward density and composition over time.

The forage breeder must always be mindful whether a particular trait has a high, moderate, or low genetic correlation between spaced plants and swards. Some complex traits such as biomass yield per unit land area usually cannot be directly translated from spaced plants to swards (Casler et al. 1996, Wilkins and Humphreys 2003). There are some notable exceptions in which selection for increased forage yield of spaced plants led to increased sward-plot yields, but there are also numerous failures to improve sward-plot yield by this approach. In an effort to compromise and employ some level of competition, many breeders utilize very narrow plant spacings, which work well for non-rhizomatous species or spaced-plants-in-swards, in which a contrasting species is overseeded to provide ground cover and a uniformly competitive environment.

The ultimate validation is to evaluate progress from selection from spaced-plant nurseries in realistic sward plots using the original population as a control. Using this process, the use of spaced-plant nurseries has been validated for many traits. Of these, some of the most reliable traits include simple morphological or physiological traits (e.g., flowering time, leaf size and shape, stem diameter, and plant height), forage quality traits, pest resistances, and some stress tolerances (see reviews by Abberton and Marshall 2005, Casler et al. 1996, Casler and Pederson 1996, Humphreys 2005, Wilkins and Humphreys 2003).

2.2 Seed and Seedling Traits

Many forage crops, particularly warm-season grasses, are difficult to establish, in some cases caused by inherently low seedling vigor and competitive ability and in other cases by environmental issues such as heat and drought during germination and establishment. Few forage crops have been domesticated in the sense that cultivars are easily differentiated from wild populations or that cultivars cannot survive without human inputs. As such, many forage crops still possess traits of wild plants that include seed shattering, small seed size, seed dormancy, and relatively slow germination rates, although some exhibit weedy traits such as rapid germination under most circumstances (e.g., *Lolium multiflorum* Lam.).

Seed size is moderately to highly heritable and readily amenable to selection. Seedling vigor is highly correlated between laboratory and field tests allowing the use of simplified laboratory or glasshouse selection protocols with large population sizes and uniform germination, emergence, and growing conditions. Larger or heavier seeds generally have increased seedling vigor, more rapid germination, larger seedling shoot mass, and more rapid adventitious root formation. Generally, the effect of larger seeds lasts for only a few weeks after germination with minimal effects on long-term growth of adult plants. Selection for rapid germination can also be used to reduce the proportion of dormant seeds in forage populations. Selection for reduced seed dormancy can be complicated by multiple mechanisms of dormancy including seed morphology, seed mass, thickness of lemma and palea, and genetic variation that is independent of seed morphology (Jones and Nielson 1999).

In addition to direct selection for seedling traits, selection on seedlings is an essential component for development of efficient DNA marker selection protocols. DNA markers can be rapidly and meaningfully screened on thousands of seedlings, based on marker-trait associations from phenotypic and genotypic evaluations of parental plants. Seedling screens of DNA markers, using adult-plant marker-trait associations provide a mechanism to apply selection pressure for complex traits such as biomass yield both among and within family pedigrees.

2.3 Reproductive Development

Forage plants undergo fundamental phase changes as they develop. Individual shoots proceed from the embryonic phase to three postembryonic phases: postembryonic development, juvenile, and adult (Poethig 2003). The transition from one phase to the next is controlled by an array of independent signal transduction pathways that respond to external stimuli such as thermal time (growing degree days) and photoperiod. During their juvenile phase, forage plants are generally highly susceptible to exogenous stresses such as drought, cold, and heat, largely because they do not yet possess the hardening mechanisms required to attain their genetic potential for stress tolerance. In grasses, there is a gradual increase in leaf blade length, leaf blade width, and leaf sheath length during the juvenile phase. Timing of the juvenile–adult transition is under genetic control (Basso et al. 2008).

The transition from vegetative to flowering phase, which generally occurs only in adult plants, is regulated by genes that belong to one of four parallel pathways – gibberellin, autonomous, vernalization, and light dependent – plus an integration pathway (Cockram et al. 2007). Many of the genes involved in flowering pathways play no role in developmental regulation during vegetative phase changes. Indeed, alterations to flowering-time genes may drastically alter flowering dates, but have no effect on timing of vegetative phase changes.

Flowering time in forage crops is a highly heritable trait, controlled by genes with additive effects and easily amenable to phenotypic selection. Genes that regulate the photoperiodic control of flowering time are highly conserved across diverse species. Identification of numerous single-nucleotide polymorphism (SNP) DNA markers within genes that control flowering creates opportunities to efficiently employ marker-selection methodologies for selection on heading date well before the trait is phenotypically expressed.

2.4 Persistence

Persistence of perennial forage crops should not be considered as a single trait, but rather as a complex of traits that are each dependent on the environment and agricultural context of the crop. The greatest achievements for improved persistence of forage crops have arisen from clear definitions of the problem and a clear path toward the solution. When perennial forages demonstrate a significant lack of persistence, experiments should be undertaken to determine the cause(s) of plant mortality. Are plants dying due to disease, insects, abiotic stresses, or stress that arises from some new management regime? Once the principal source of plant mortality is identified, a selection protocol or phenotypic screen should be designed to allow plants to be uniformly exposed to the stress(es). Can this be done in the laboratory or glasshouse to improve uniformity and repeatability, or must it be done in the field for realistic assessment? Can the phenotypic screen be applied to seedlings with a reasonable expectation of selecting plants with improved adult-plant persistence?

Bacterial, fungal, and viral pathogens, nematodes, and insect pests are frequent causes of mortality in forage plants, particularly those that affect the vascular system and/or roots of the host plant. There are numerous examples of improved persistence directly resulting from selection and breeding for pest resistance (see reviews by Abberton and Marshall 2005, Casler and Pederson 1996). In most of these examples, selection protocols have been based on uniform screens of thousands of seedlings or juvenile plants, often evaluated under artificial inoculations in the laboratory or glasshouse.

Abiotic stresses also play an important role in limiting the persistence of some forage species. Mortality can be caused by abiotic stresses such as heat, cold, drought, flooding, acid soils, salinity, heavy metals, air pollutants, and severe management regimes. Morphological traits, such as stolon, rhizome, or root characteristics, can be manipulated to reduce persistence problems caused by some abiotic agents such as heat and drought (Abberton and Marshall 2005, Casler et al. 1996). Many populations of forage plants may contain very low frequencies of genes for tolerance to one or more abiotic stress factors, challenging the breeder to design a selection protocol that will capture the few plants that possess these genes and to then concentrate them in resistant or tolerant populations.

Many perennial grasses and some legumes are host to endophytic fungi that form mutualistic relationships with their host. The fungi obtain water, nutrients, and a long-term survival mechanism from the host, at the same time producing alkaloids and other compounds that protect the host from herbivory and some abiotic stresses. Fungal endophytes (*Neotyphodium* spp.) associated with tall fescue (*Festuca arundinacea* Schreber) and ryegrasses (*Lolium* spp.) produce ergot alkaloids that are responsible for severe health problems in bovines resulting in huge economic losses for cattle producers. Because endophytic fungi can protect host plants from several stress factors, the presence or absence of the endophyte may drastically alter the breeder's objectives in some environments and managements (Pedersen and Sleper 1988). Isofrequent endophyte-containing and endophyte-free populations can almost be considered as different species as far as breeding objectives are concerned. Newer approaches to dealing with the endophyte problem in fescues and ryegrasses include deliberate infection with strains with drastically lower ergot alkaloid production and even strains where key enzymes in the ergot pathway have been knocked out.

3 Biomass Yield and Its Components

3.1 Measurement of Biomass Yield

Biomass yield is most appropriately measured on sward plots, established either as drilled rows or from broadcast seed. Where seed is limiting, closely spaced plants can be used to simulate sward plots provided that the spacing is sufficiently narrow to allow interplant competition and sufficient time is allowed for plants to spread into each other, filling the open spaces. Caution should always be used for inferences from sward plots established from closely spaced plants, because these plants represent random plants of the population or family being evaluated. Mortality rates are high in sward plots established from seed – up to 90% mortality during the establishment year – and strong selection pressure for fitness traits has been observed within the first 2–3 years after stand establishment. Due to mortality and selection, the genetic and phenotypic composition of both grass and legume sward plots can change rapidly so that sward-plot evaluations of families or breeding lines are generally not evaluations of random plants from a bag of seed. Other methods of assessing biomass yields include row plots or spaced plants, using a fairly wide spacing between rows or plants, but the potential pitfalls of widely spaced plants were discussed earlier.

The size of sward plots varies considerably among forage breeding programs, usually determined by the seeding and harvesting equipment available. Because seed of families or breeding lines is generally limiting, plot sizes for family selection protocols are generally smaller than for cultivar evaluations. With an appropriate experimental design and statistical analysis, small sward plots (1.2 m^2) can be very effective for half-sib family evaluations of breeding value.

Experimental designs for sward-plot evaluations of families can range from simple randomized complete blocks to a wide array of incomplete block designs. The latter group includes blocks-in-reps (a variation of the split-plot), many types of lattice designs, and α-designs. Because blocking designs rely on the breeder's ability to predict patterns of field variation and lay out blocks accordingly, postdictive spatial analyses are often useful supplements to experimental design for controlling unexplained spatial variation and providing accurate estimates of family means. Trend analyses or nearest neighbor analyses have been simplified within mixed models frameworks so that they can be easily incorporated into the analysis of many families evaluated over multiple years and locations (Smith and Casler 2004).

3.2 The Role of Environment and Management in Measuring Biomass Yield

Biomass yield is one of the most important traits of forage plants, acting as the single unifying trait that is measured in nearly every cultivar evaluation trial, regardless of the environment or agricultural context. The most appropriate measurement of biomass yield depends on the agricultural context of the forage crop. Management schemes may range from one conservation harvest per year for warm-season bioenergy grasses to continuous stocking of grass–legume pastures, including nearly any intermediate timing and frequency of cutting or grazing. For conservation managements, a machine harvester is generally used to harvest forage plots, cutting the crop with a sickle-bar mower or a flail chopper. Harvest managements and timing will vary among species and location, but should always be designed to accurately reflect real-world practices in the target region for the forage crop. The old breeder's axiom should always be remembered, "What you select is what you get."

Conversion of fresh-matter forage yields to dry-matter forage yields is problematic for large replicated trials containing hundreds of families and multiple locations, cuttings, and years. For forage species or breeding populations of the same ploidy level and in which maturity is relatively constant, fresh-matter and dry-matter forage yields are highly correlated so that fresh-matter forage yield can be used as a surrogate for dry-matter forage yield. This decision must be made with great care and thought, because genetic variation for growth stage or flowering time could confound the breeder's ability to make genetic progress when dry-matter concentration is negatively correlated with fresh-matter forage yield.

The methodology for measurement of forage yield under grazing management is much more variable than under conservation management. Grazing managements are often simulated by machine-harvesting sward plots on a schedule that simulates local grazing managements. However, simulated grazing management by frequent machine harvesting captures only the first of four major effects that grazing livestock may exert on pasture plants: (1) frequent defoliation and the requirement for frequent regrowth and regeneration, (2) selectivity of plants that may favor persistence of specific genotypes, (3) trampling that may damage plant tissue and compact the soil, and (4) excretion that concentrates nutrients in small areas affecting palatability and nutrient cycling (Hart and Hoveland 1989). The last three of these effects create the need for larger plot sizes and more replicates than typically used for large-scale family evaluations under conservation management, largely because of the additional within-plot variability induced by livestock. In a rotational grazing scheme, higher stocking rates and shorter grazing times can be used to increase uniformity of grazing management within and among breeding plots.

Under grazing management, herbage mass can be measured directly (destructive sampling) or indirectly (nondestructive sampling). Direct measurement of herbage mass involves the use of a quadrat or sampling frame and hand harvesting all herbage within the frame to a specific cutting height at or below the desired grazing height. For estimates of total herbage mass, cutting height should be at the soil surface. Indirect measures of herbage mass are classified into three groups: visual, height and density, and nonvegetative attributes. For quantification of herbage mass, all indirect measures must be calibrated by the use of a double-sampling technique, preferably repeated over time and space to capture environmental variation.

Employing the previously mentioned breeder's axiom, it should be obvious that different plants will be selected under different management schemes, depending on the traits that favor persistence, recovery, stress tolerance, and/or high herbage mass. Empirical studies have demonstrated that there is often little or no correlation between biomass yield measurements under highly divergent management schemes, particularly for management schemes that place different stresses upon the plants such as cutting versus grazing, i.e., genotype × management interaction. Many breeders have attempted to develop grazing-tolerant alfalfa (*Medicago sativa* L.) by either trait selection or selection of plants under frequent mowing – all of these efforts failed to produce a grazing-tolerant alfalfa. In contrast, selection of alfalfa plants that survived several months of continuous stocking resulted in germplasm with consistently high grazing tolerance under both continuous and rotational stocking. High forage yield of the grazing-tolerant alfalfa cultivars under conservation management indicates that the addition of the grazing tolerance trait has broadened the adaptation of this germplasm (Bouton and Gates 2003).

Because most forage crops are grown in mixed swards, often including at least one grass and legume species, there has been considerable effort to define how best to select plants for competitive ability or combining ability with companion species, sometimes referred to as coexistence. Trait breeding in pure swards – flowering date, tiller density, canopy height, and leaf traits (leaf length, leaf size, petiole length) – may be effective for developing either grass or legume germplasm compatible with companion species in mixed swards. Alternatively, strong interactions between genotypes and sward types (pure versus mixed swards) in many studies, suggesting complex mechanisms that regulate competition for space, validate the breeder's axiom that selection for performance in mixed swards should be conducted in mixed swards.

3.3 Breeding for Increased Biomass Yield

Gains made in biomass yield of forage crops vary widely among regions and species, generally ranging from about 1 to 6% per decade (Humphreys 1999, Wilkins and Humphreys 2003) but gains may be dependent on environment and management. Pyramiding multiple pest resistances is a mechanism for improving persistence of alfalfa, but decades of breeding for multiple pest resistances in alfalfa resulted in no change to biomass yield potential in the absence of significant disease pressure (Lamb et al. 2006). Similarly, significant gains made in biomass yield under hay management may not be observed under management-intensive rotational grazing, due to the absence of livestock during the selection process.

Gains in biomass yield of forage crops have largely resulted from selection of superior families and recombination of parental clones or selected plants of the best families. (Details of family-based breeding schemes are provided in Chapter 3.) Intensive selection for increased biomass yield of sward plots can lead to rates of gain as high as 10% per decade (Wilkins and Humphreys 2003). However, family selection based on sward plots carries a significant cost in the relatively large number of families that must be continually carried through the breeding program and the large number of plots that must be repeatedly harvested. For those species in which spaced-plant and sward-plot biomass yields have a positive genetic correlation, phenotypic selection of spaced plants can be used to effectively increase biomass yield of sward plots. Generally, gains measured in sward plots are generally reduced to half or less than those observed on spaced plants, because selection was conducted under non-competitive conditions and the genetic correlation between competitive and non-competitive conditions is less than one.

Indirect selection has often been employed to improve biomass yield per se, but none of these efforts have led to long-term sustained gains in biomass yield. Some of the traits that have shown promise as indirect selection criteria for biomass yield of forage grasses include specific leaf weight, leaf length, leaf area expansion rate, mesophyll cell size, and dark respiration rate (Casler et al. 1996). Indirect selection for stolon and leaf traits of white clover has probably had some positive impact on biomass yields of white clover (Abberton and Marshall 2005, Woodfield and Caradus 1994). The general conclusion is that if one aims to improve biomass yield one should select based on biomass yield, which is only restating the breeder's axiom mentioned above in a slightly different form.

3.4 Seasonal Distribution of Biomass Yield

Improved seasonal distribution of biomass yield has long been a goal of forage breeders and agronomists. Extension of the growing season, either by early-spring growth or late fall growth, or more uniform production throughout the growing season has been the most common target. Non-uniform dry-matter production throughout the growing season is typically tied to reproductive growth – plant biomass increases up to a certain point associated with flowering, when seed formation, remobilization of storage carbohydrates, and leaf senescence lead to reductions in plant biomass. For cool-season forages, particularly grasses, this results in a "summer slump" in which biomass production is significantly reduced during the warmest period of summer, often to the point of dormancy for some species.

Early flowering types of cool-season grasses and legumes have a more even distribution of dry matter throughout the growing season creating a longer post-flowering growth period in humid, temperate regions. Non-flowering or sparse-flowering populations have been developed in some forage grasses with the goal of increasing nutritional value, simplifying grazing management, and creating a more favorable seasonal distribution of forage yield compared to normal-flowering populations. In timothy (*Phleum pratense* L.), early-heading genotypes with a high frequency of flowering tillers on regrowth harvests had the highest biomass yields in summer and for the entire growing season, resulting in a more uniform distribution of biomass production.

4 Nitrogen Economy

4.1 Nitrogen Fixation of Legumes

Forage legumes fix atmospheric nitrogen (N_2) as a source of internal nitrogen nutrition and as sources of nitrogen nutrition for companion grasses or a subsequent crop in a crop rotation system. N_2 fixation can be measured directly by the acetylene-reduction assay, the difference method (total reduced N of a treatment versus a non-N_2-fixing control), or the ^{15}N isotope dilution method. The acetylene-reduction method has been used to develop alfalfa genotypes and cultivars with significantly improved levels of N_2 fixation, repeatable in multiple field environments. This research has led to the development of alfalfa cultivars with high N_2 fixation and biomass yield for use specifically as an annual plow-down crop in crop rotations. Indirectly, N_2 fixation can be improved by selection for high tissue N concentration in inoculated legume plants grown on a N-deficient soil. Recent research with ^{15}N isotope dilution indicates that the application of N-fertilizer to mixed white clover (*Trifolium repens* L.) – perennial ryegrass (*Lolium perenne* L.) stands increases N-transfer from the legume to the grass. The mechanism is greater rooting in the grass component and hence, better access to the N deposited by legume roots; N-fixation but not exudation (ammonium and amino acids) is depressed by N-application (Paynel et. al, 2008).

4.2 Nitrogen Use Efficiency of Grasses

Nitrogen use efficiency (NUE) can be improved in grasses by selection for increased biomass yield under uniform soil-N conditions. The levels of soil-N and applied fertilizer should be closely aligned with the intended agricultural context of the crop. If the crop is to be grown under conditions of relatively low N nutrition, as is often the case with perennial grasses, selection should be conducted under these conditions to identify plants capable of scavenging N from these soils. Under these conditions, increased biomass yield will arise from a reduction in tissue N concentration and/or an increase in the proportion of available soil N recovered by the plants (Wilkins and Humphreys 2003). Tissue N concentration is moderately to highly heritable in perennial grasses, so selection for combined high biomass yield and high tissue N concentration may be an effective method of indirect selection for more efficient N uptake as a mechanism to improve NUE.

5 Forage Quality

5.1 Laboratory Estimators of Forage Quality

Breeding forages with improved quality requires laboratory assays that are rapid, repeatable, heritable, and are directly correlated with animal performance. *In vitro* dry-matter digestibility (IVDMD), including various modifications of the original Tilley and Terry (1963) procedure, was the first of these assays to receive overwhelming support from the scientific community. Breeding efforts for increased IVDMD were initiated almost immediately after publication of the Tilley and Terry procedure. The in situ nylon bag dry-matter digestibility (NBDMD) procedure was one of the earliest and most highly successful modifications of the original assay. The attractiveness of the IVDMD procedure derived largely from its direct reliance on rumen microorganisms for tissue degradation and its role in ruminant nutrition, directly through *in vivo* digestion and indirectly through a positive impact on voluntary intake.

Traits such as IVDMD are determined by fundamental chemical and physical traits of the plant, including cell-wall structure and composition, anatomical composition of organs, and organ morphology. Four mechanisms to increase IVDMD of forage plants have been demonstrated: (1) decreased fiber or cell-wall concentration, (2) increased concentration of water-soluble carbohydrates (WSC), (3) decreased lignification of the cell wall, and (4) decreased ferulate cross-linking between lignin and polysaccharides in the cell wall (Casler and Vogel 1999, Casler 2001). The first two of these mechanisms probably overlap with each other – without an increase in net photosynthesis there is a fixed carbon pool that is allocated between the soluble and structural carbohydrate pools, theoretically resulting in a negative correlation between structural and soluble carbohydrate concentrations. The latter two mechanisms operate independently of each other, both acting to increase IVDMD and, more importantly, digestibility of the fiber or cell-wall fraction per se.

Laboratory measures of intake potential have been much more problematic for forage agronomists and breeders, largely because voluntary intake measurements are more susceptible to inherent variability and are less directly related to measurable plant traits. The concentration of neutral detergent fiber (NDF), a simple and inexpensive assay, has long been considered as the single laboratory variable most directly related to voluntary intake of ruminants (Casler and Vogel 1999, Casler 2001). Other measures of intake include physical measurements such as particle-size breakdown by ball milling or artificial mastication, the energy required to grind a forage sample to pass a given mesh size, and the energy required to shear an intact leaf blade, respectively (see reviews by Casler and Vogel (1999) and Casler (2001).

5.2 Breeding Methods and Breeding Progress

Most laboratory traits that predict forage quality are highly stable and repeatable across a wide range of environmental conditions. They tend to be far less sensitive to genotype × environment interactions than agronomic traits such as forage yield and stress tolerances. Most breeding activities begin with a spaced plant nursery, allowing easy harvest, identification, and eventual recovery of individual plants. Samples are collected from each plant, usually by hand harvest, in a uniform manner and processed as uniformly as possible throughout the stages of drying, grinding, and analysis. Traditional methods of analysis would involve direct assay of each sample by wet laboratory methods, but that process has been largely streamlined by the use of near-infrared reflectance spectroscopy (NIRS). In most breeding programs, samples are scanned using NIRS and a broadly representative subset of samples is analyzed, using wet chemistry methods. Prediction equations are developed from the calibration set and used to generate predicted values for the entire breeding nursery.

The use of NIRS in a breeding program is highly cost-effective, reducing the need for wet chemistry to a very small proportion of the total samples, sometimes as low as 10%. Calibration equations can be developed using either open or closed population calibration methods. Open population calibration involves development of a broad calibration set, typically for one species or group of similar species, with occasional new samples added to the calibration set to represent new environments, genotypes, or growing conditions. Closed population calibration involves development of calibration sets for specific experiments or selection nurseries, with no attempts to develop broader calibrations across space or time. As NIRS technologies, statistical methods, and software have improved, open population calibration methods have become more accurate and precise and are gradually replacing closed population methods in many breeding programs.

Once the most favorable plants are identified, they are typically removed from the original nursery and transplanted to crossing blocks in the field or in isolated crossing houses. Crosses can be made between individual plants with superior levels of a trait or among a larger group of plants, depending on the breeding methods being employed. In a recurrent selection program, the resulting seed is used to establish a new nursery to begin the next generation or cycle of selection.

Two critical factors have simplified many breeding schemes designed to improve quality of forage crops. First, heritability of many forage quality traits (e.g., IVDMD, NDF, WSC, crude protein, plus many others) is moderate to high for selection on the basis of an individual unreplicated plant. This principle has been verified many times, most effectively by completion of one or more cycles of selection for a trait, followed by evaluation in a new series of experiments, typically under sward-plot conditions. The moderate to high heritability of most forage quality traits has been verified by positive and significant genetic gain, as indicated by superiority of a new population compared to the original population from which it was derived, in a new experiment under different growing conditions from the original nursery (typically sward plots versus spaced plants). Second, genetic gains made for most forage quality are stable under a wide range of conditions, including different growing conditions, growth stages, harvest managements, and soil types. Realized gains for WSC and IVDMD of perennial ryegrass have demonstrated stability from spaced plant nurseries to sward plots to on-farm trials under grazing livestock (Humphreys 1989, Walters 1984).

Genetic gains in IVDMD, WSC, and other traits related to digestibility and intake potential of forage crops may accrue very rapidly, with published results indicating gains of 5–66 g kg^{-1} $cycle^{-1}$ (Casler 2001). Most selection programs are capable of completing a cycle of selection within 2 or 3 years, depending on the vernalization requirements of the species, the need to assess stress tolerances or fitness of the selected plants, and the amount of seed required for the next generation of selection. The use of significant modifications to the selection methods and personnel dedicated to this effort at critical times may allow a reduction in generation time to 1 year for species that can be forced to flower within that time and for which there are no stress tolerance or fitness issues.

Despite the ready availability of efficient selection criteria that are related to animal performance and have excellent laboratory repeatability, relatively few breeding programs are dedicated to improving forage quality. For forage crops with relatively high inherent quality other traits and objectives demand the most attention. In other cases, there are still many perceptions that breeding for forage quality is a sure way to decrease forage yield and other fitness traits of forage crops. Thorough review of the literature indicates that fitness problems associated with genetic improvements in forage quality are very much the minority (Casler 2001). There have been reports of reductions in lignin concentration associated with decreased cold tolerance and increased disease susceptibility, but other reports have shown that these problems can generally be overcome by concomitant selection for fitness traits in the field to ensure that these traits are not lost during the selection process. Among forage quality traits that are controlled by multiple genes with relatively small individual effects, what we normally describe as quantitative genetic variation and quantitative trait loci (QTL), only NDF has shown consistent fitness problems across multiple populations and species. Reduced NDF has been consistently associated with reduced forage yield and detailed genetic analyses have indicated that this is due to multiple causes that include genes with multiple specificities, linkage between genes controlling both traits, and random processes such as drift (Casler, 2005).

5.3 Anti-nutritional Factors

Many types of plant compounds and structures can be detrimental to utilization of forage crops by livestock, largely by reducing palatability, digestibility, intake, and/or health and fitness of livestock. These traits exist largely as defense mechanisms in plants that have coexisted with mammalian herbivores for eons, all designed to reduce herbivory, while herbivores evolved traits to overcome these defense mechanisms, including mouth and tooth structure, rumination, multi-chambered fermentation systems, and diverse microbial populations.

Most defense mechanisms are chemical in nature, although there are examples of physical or structural defense mechanisms, such as trichomes or siliceous dentations on leaves or stems. Chemical defense mechanisms include toxins, estrogenic compounds, and narcotic compounds, such as alkaloids. Alkaloids and cyanogenic compounds that contain nitrogen may serve the dual roles of defense against herbivory and sequestration of nitrogen. Saponins, tannins, estrogens, and cyanogenic compounds are common in many important forage legumes. While tannins are known to cause reductions in palatability of some legumes, they are also thought to play an important role in binding soluble proteins in the rumen, helping to protect ruminants against bloat. Genetic variability and relatively simple inheritance patterns have been demonstrated for many of these compounds, which are fairly amenable to selection. Selection for low levels of the estrogen formononetin in red clover (*Trifolium pratense* L.) resulted in significant improvements in fertility and breeding times of ewes (*Ovis aries*) (McDonald et al. 1994). Condensed tannins in sericea lespedeza [*Lespedeza cuneata* (Dum. Cours.) G. Don] and sainfoin (*Onobrychis sativa* L.) confer two advantages to small ruminants: control of internal parasites such as *Haemonchus contortus* and increased bypass protein, both resulting in increased animal performance.

Natural populations of reed canarygrass and phalaris (*P. aquatica* L.) contain a wide array of alkaloids that can have toxic and narcotic effects on livestock, reducing palatability, intake, digestibility, health, and fitness. Two genes control the type of alkaloid, simplifying the process of identifying and intercrossing true-breeding plants without the highly toxic compounds in the tryptamine and β-carboline chemical families. The less-toxic alkaloid, gramine, causes little more than reductions in palatability, which have been solved by screening large populations of individuals for gramine concentration in the process of producing low-gramine cultivars. Grazing trials have demonstrated that elimination of tryptamines and β-carbolines reduces animal disease issues, while reductions in gramine concentration increase palatability, intake, and liveweight gain (Marten 1989).

Mineral imbalances, such as inadequate or excessive levels of one or more mineral elements, can be classified as anti-nutritional factors without necessarily being anti-herbivory defense mechanisms. Hypomagnesemia is the most serious disease caused by a mineral imbalance, specifically a deficiency of Mg or an excess of K in fresh herbage. Because mineral elements such as Mg and K are under genetic control, selection for increased Mg and/or reduced K concentrations have led to the development of new cultivars with reduced potential for this disease. Grazing studies have demonstrated significant gains in blood serum Mg and reductions in livestock fatalities in direct comparisons of high-Mg versus low-Mg cultivars (Moseley and Baker 1991).

5.4 Livestock Evaluations

A demonstration of increased animal performance is the definitive proof of impact from breeding forage crops for increased forage quality. As mentioned above, this has been accomplished in several instances of selection against anti-quality factors. Yet, although there are few reports of successful validation from grazing or feeding trials, there is convincing evidence that genetic increases in IVDMD, WSC, or related traits will generally positively impact livestock performance (Casler and Vogel 1999).

Increased IVDMD has a direct and positive impact on average daily weight gains of livestock. This effect is typically greatest for warm-season grasses which generally have the lowest forage quality, providing a greater potential benefit for the same investment in selection and breeding. Because most new cultivars with genetic increases in forage quality also represent increases or no change in forage yield, relative to their parent cultivars, increased liveweight gains per animal typically translate to increased liveweight gains per hectare. Despite their expense, grazing and/or feeding trials have a proven benefit in supporting efforts to market and distribute new cultivars. The three most successful forage breeding programs dedicated to improving forage quality for at least 25 years – the USDA-ARS bermudagrass [*Cynodon dactylon* (L.) Pers.] program at Tifton, GA, USA; the USDA-ARS warm-season grass program at Lincoln, NE, USA; and the UK ryegrass program at Aberystwyth, Wales – all rely on grazing experiments to document the effect on livestock performance. Often, these animal performance data are largely or solely responsible for the success of new cultivars.

6 Biotic and Abiotic Stresses

6.1 Breeding for Durable Pest Resistance

The perennial nature of many forage crops, the use of many species in monocultures, and the broad range of environmental conditions over which many forage species are grown all contribute to pest problems. Many of these pests reduce forage yield and/or quality, may contribute to reduced livestock performance or health, and result in reduced vigor/significant mortality of forage plants. Breeding for pest resistance is perhaps one of the most common objectives in forage breeding. It could be argued that all forage breeders participate in this activity, at a minimum by culling highly diseased plants within selection nurseries, even when disease resistance is not the primary breeding objective. In alfalfa and red clover, genetic gains for multiple pest resistances represent the most significant historical breeding progress, leading to improvements in persistence and stand longevity, forage yield, and economic returns (Elgin 1985, Smith and Kretschmer 1989).

Phenotypic recurrent selection is by far the most common selection method used to improve pest resistance in forage crops. This selection process involves development of a uniform screening process, ensuring that all plants are grown in a uniform environment and that pathogen inoculum is uniformly delivered to each plant. Many of the largest forage breeding programs will routinely screen over 1 million plants per year for disease resistances.

For these programs, and for many smaller programs, the use of indirect selection methods is an absolute requirement. Indirect selection involves selection for a trait that is not exactly the target trait, but is positively correlated with the target trait to such a degree that the losses associated with selection for a different trait are more than offset by the gains associated with improved selection efficiency and intensity. For example, phenotypic selection for increased yield will invariably lead to the elimination of susceptible genotypes for temperate forages grown in a subtropical humid environment. The use of seedlings that may still be in a juvenile developmental phase and glasshouse screening environments create highly uniform, but artificial, conditions for pathogen development. Nevertheless, uniformity of both the environmental conditions and the delivery of pathogen inoculum, combined with the potential to screen many more genotypes than could be screened in the field, has resulted in realized genetic gains for many pathogens of many species (Casler and Pederson 1996). While there are many success stories, there are some examples in which seedling screens in the glasshouse were either not successful or less efficient than field selection, due to low correlations between the glasshouse and the field and/or seedlings and adult plants. Research on these relationships should be undertaken in all circumstances to determine the most efficient selection methods and the trade-offs associated with indirect versus direct selection. Indirect selection for forage yield or vigor in the presence of uniform pathogen loads can be a very effective method of improving resistance to viruses or nematodes in forage populations.

Phenotypic recurrent selection does not require the presence of "resistant" plants in the population targeted for improvement. Resistance genes may often be present as hidden recessives in extremely low frequencies, leading to the presence of clearly resistant plants only after two or three cycles of selection have increased the frequency of resistance alleles in the population and created plants homozygous for these alleles. Once the first resistant plants appear in a population, the rate of progress may rapidly increase due to selection of a higher frequency of resistant plants each of which carry higher doses of resistance alleles.

Many pathosystems are governed by host–pathogen specificity in which gene-for-gene relationships regulate the disease phenotype and host genotypes do not respond uniformly to all isolates or races of a pathogen. It is clear from thorough review of the literature that these gene-for-gene relationships exist in many forage pathosystems, but it is also clear from the many successful selection experiments that selection for resistance to a single pathogen isolate or a simple mixture of a small number of isolates leads to success in the vast majority of cases (Casler and Pederson 1996). The goal in most breeding programs is to utilize isolates that are present in field conditions and are highly pathogenic to the host, placing maximum selection pressure on the host population without killing all host plants.

Genetic regulation of host resistance varies from single major genes to oligo- or polygenic resistance. In theory, multiple-gene resistance should be more durable than single-gene resistance. A number of examples of long-term durability of single-gene resistance exist within forage pathosystems (Casler and Pederson 1996). Because most forage species are highly self-incompatible, largely outcrossing, and composed of highly heterogeneous populations of highly heterozygous host plants, a tremendous amount of genetic diversity is maintained within most cultivars. This is particularly true for polyploid species. Breeding for increased pest resistance increases the frequency of resistance alleles, resulting in frequencies of resistant plants of 30–60%, seldom more than 70%, in finished cultivars. These percentages are typically sufficient to withstand moderate mortality rates that occur due to pathogens and other stresses, while resistant plants are capable of compensatory growth to help fill in empty spaces created by mortality of susceptible plants. Such a system allows some level of coexistence between the host and pathogen, reducing selection pressure on particularly virulent strains of the pathogen that may arise from mutation or genetic recombination, leading to some very durable host resistances.

6.2 Moisture Stress

Drought tolerance is a very elusive trait in many species, without obvious phenotypic variation upon which to base selection. While it may seem to be the simplest and most obvious approach, simply withholding or reducing water for a certain time period, either in a glasshouse or with field-based rainout shelters, often fails due to lack of uniformity in application of drought, low heritability, or inability to predict a drought regime to optimize selection pressure.

Many approaches to improve drought tolerance have utilized indirect selection. Some of the most promising of these traits include high stomatal resistance (less frequent stomata, smaller stomata, or shallow epidermal ridges), low leaf water conductance, low osmotic potential, and low C isotope ($^{13}C/^{12}C$) discrimination ratio (Casler et al. 1996, Johnson and Asay 1993). Selection experiments for these traits have led to populations of plants with potentially improved drought tolerance, assessed as increased water-use efficiency or soil moisture levels, but these efforts have not yet led to commercial cultivars with improved drought tolerance.

Many programs have specifically targeted germplasm collection from regions that have historically been drought prone, hoping to capitalize on hundreds or thousands of generations of natural selection for drought tolerance. North African populations of perennial ryegrass appear to offer some potential in this regard. Directed natural selection also offers some promise as a viable approach, in which selection is based on long-term survivorship of plants on drought-prone soils. A combination of methodical and natural selection methods has been used in one of the most successful selection programs for drought tolerance of grasses used in dryland agriculture of western North America. Selection for rate of emergence from deep planting (5 cm) has been successful in several species, with positive correlated responses for emergence percentages and first-year forage yields in the establishment year for multiple field sites.

Interspecific hybrids and trait introgression are a mechanism of transferring drought tolerance from one species into a more agronomically or nutritionally desirable species. Transfer of drought tolerance from Kura clover to white clover and from meadow fescue (*Festuca pratensis* Huds.) to perennial and Italian ryegrass (*L. multiflorum* Lam.) have created populations with improved drought tolerance and many of the traits of the desirable parent (Abberton and Marshall 2005, Thomas et al. 2003).

Susceptibility to flooding can be caused by two factors: the direct effect of anoxia or susceptibility to oxygen deprivation and/or the indirect effect of increased pathogen load (increased inoculum loads, unique pathogen species, or increased pathogen diversity) in chronically wet soils. Forage breeders routinely use chronically wet soils known to have high pathogen loads to utilize a combination of natural and methodical selection for resistance to these pathogens and, indirectly, increased tolerance to wet soils. Most research on flooding tolerance of forage crops has focused on species selection, with a limited amount of research demonstrating genetic variation among cultivars and genotypes for flooding tolerance.

6.3 Temperature Stress

Cold or freezing tolerance is one of the most important factors limiting the adaptation and long-term survival of many forage crops. Low-temperature stress interacts with other stresses, such as grazing pressure, snow molds, drought, and dessicating winds, often complicating the development of efficient screening procedures. Furthermore, low-temperature stress is conditioned by hardening plants to lower temperatures, while winter or early-spring thaws can deharden plants to reduce their low-temperature stress tolerance. Snow cover also interacts with low-temperature stress tolerance by protecting plants from severe cold. Forage germplasm with superior low-temperature stress tolerance often can be found in climates with severe winter temperatures, except where snow cover is frequent and consistent.

Several mechanisms for improving low-temperature stress have been identified in forage crops. Enhanced resistance to winter diseases such as snow molds and some root or crown rot organisms have resulted in healthier plants that are better able to withstand low-temperature stress. Genetic increases in WSC may enhance low-temperature stress tolerance, partly as a direct effect of WSC on spring recovery and partly as an indirect effect of WSC on tolerance to snow mold fungi. Unsaturated fatty acids and vegetative storage proteins have also been implicated as potential mechanisms for enhancing low-temperature stress tolerance in perennial forages. Photoinhibition can directly cause low-temperature stress, occurring during periods of high light and low temperature when the rate of light harvesting by PSII exceeds the capacity for electron transport. Androgenic plants derived from *Festuca pratensis* × *L. multiflorum* hybrids have revealed multiple genetic pathways that reduce the effects of photoinhibition during low temperatures (Rapacz et al. 2004). Morphological traits, such as stolons and rhizomes, may also act as a mechanism to enhance low-temperature stress tolerance in some perennial forage species.

Selection for low-temperature stress tolerance can be carried out effectively under artificial conditions. Plants must be systematically hardened prior to the onset of stress and these tests typically require a range of temperatures to accurately assess the potential range in responses within a diverse population. Selection is often based on LT_{50}, the temperature predicted to kill 50% of the tillers on a plant. In many perennial forage species, juvenile plants are incapable of undergoing cold hardening, forcing breeders to screen adult plants. Selection for low-temperature stress tolerance of seedlings may improve seedling tolerance levels, but it will likely have little impact on adult-plant tolerance levels (Hides 1979). Because low-temperature stress screening can be highly variable, plants are often vegetatively propagated or cloned for replicated screening of genotypes in time or space.

Tolerance to high temperatures can be identified using either field- or glasshouse-based screening procedures. In the glasshouse, repeatability, uniformity, and reproducibility of the screening procedure can be enhanced by the use of heating elements embedded in sand benches. While it is not documented per se, the gradual movement of cool-season species such as tall fescue, alfalfa, and red clover to lower latitudes of the southern USA has probably resulted from extensive, long-term, field-based breeding programs in Florida, Georgia, Alabama, and Texas that have selected for increased high-temperature stress tolerance, either consciously or unconsciously.

6.4 Chemical Stresses in Soils

Significant breeding efforts have been undertaken to increase acid tolerance of alfalfa, white clover, perennial ryegrass, and phalaris. Several selection protocols have yielded positive results, including (1) field-based selection on highly acidic soils, (2) relative root growth of seedlings exposed to solution culture with relatively high Al concentration, (3) relative root growth of seedlings grown in a soil-on-agar medium (seeds planted between agar and an 8-mm layer of acidic soil), and (4) transformation of alfalfa with a bacterial citrate synthase gene. Laboratory protocols tend to be preferred, because of greater control over environmental conditions, leading to increased uniformity of selection pressure, higher repeatability, and more reliable genotype assessments. A number of candidate genes homologous to known Al-tolerance genes in other plant species have been identified in alfalfa (Narasimhamoorthy et al. 2007).

Natural genetic variation exists for salt tolerance in a wide range of forage species, so breeding for salinity tolerance is generally a relatively simple matter of developing an effective screening method combined with a few cycles of selection to increase the frequency of favorable alleles and tolerant plants. The most effective screening procedures are generally based on rapid germination or rapid root growth in a saline solution, although there are a few examples of genetic variation among germplasm collections directly associated with salinity of the local environment (Casler et al. 1996). Progeny of plants selected for germination or root growth in saline solution culture generally breed true for salinity tolerance, and demonstrate improved performance, including germination, vigor, and forage yield under saline field conditions (Jensen et al. 2005). Even though genes for salinity tolerance may be present in extremely low initial frequencies within breeding populations, intensive selection pressures with large population sizes can lead to tolerant populations.

Observed tolerances to heavy metals have largely arisen by natural selection of a very small number of plants capable of surviving on toxic soils associated with mine spoils, smelters, and electricity pylons (Casler et al. 1996). Investigation of neighboring populations has routinely demonstrated frequencies of alleles for heavy metal tolerances as low as 0.0001 in some populations, sufficiently high for a very small number of plants to survive. Most heavy metal tolerances are simply inherited by a single locus so that tolerant plants typically breed true and can be used to create tolerant populations, many of which have been used to renovate contaminated soils. Because several heavy metals share similar uptake mechanisms, many genes for heavy metal tolerance have specificities for multiple elements, increasing the potential value and scope of heavy metal-tolerant populations.

7 Interrelationships Among Breeding Objectives

The ultimate choice of breeding objectives depends on many factors. Breeders must often make difficult decisions with little scientific information of direct relevance to the specific objective. Practical plant breeders are much more than people who develop new cultivars – they are problem solvers. The use of forage breeding to solve forage production problems requires sufficient scientific knowledge to identify the problem, prediction of a potential solution with a reasonably high degree of certainty, identification of reasonable and reliable breeding methods and traits, and the presence of sufficient genetic variability to create new germplasm that is sufficiently improved to assist in solving the problem.

Potential breeding objectives should be weighed against alternative solutions to production problems. Are forage producers trying to grow a particular species in an environment to which it is not adapted? If so, is it worth the breeder's time, effort, and funds to solve this problem with selection and breeding? Because genetic variability for many physiological plant traits is often hidden to us until we design the appropriate screening procedure, we often cannot predict the probability of success of new breeding objectives and ventures. In many cases, there are more cost-effective agronomic, production, or management solutions to the production problems associated with a particular species, one of the simplest being to choose different species.

Setting priorities among potential breeding objectives requires the breeder to conduct an assessment of gain versus risk, even if this is conducted informally or with little scientific input. The breeder's personal knowledge and skills, availability and talents of support personnel within the breeding program, the physical facilities and equipment available to the breeding program, and the long-term funding prospects all factor into an effective assessment of gain versus risk. Many of the plant traits discussed above have received considerable attention largely because they are moderately to highly heritable, they are simple and easy to assess, they are relatively inexpensive to measure (allowing application in multiple species and/or breeding populations), and their potential impact on forage production can be assessed with relatively simple and effective methods. Many disease resistances and some stress tolerances fit into this category. On the other hand, some complex traits such as "persistence" have required many years of effort and inputs from many scientists to develop effective screening procedures, effectively reducing the risks associated with these particular objectives in a breeding program, e.g., freezing tolerance and grazing tolerance as discussed above.

Compounding these factors, many breeding objectives are interrelated to each other in complex ways, often governed by genetic correlations that result from close linkages between genes or genes with multiple specificities. Breeding objectives that are too narrowly focused may often result in unintended consequences, such as reduced fitness associated with selection for increased forage quality (Casler 2001) or reduced root growth associated with long-term selection for increased forage yield (Gates et al. 1999). Obviously, forage breeders cannot afford to measure all traits that are potentially important in the production system of a forage crop, but selection systems should be designed to minimize the effects of random genetic drift, relaxation of selection pressure for fitness-related traits, and known genetic correlations with potentially negative production implications. Supplementation of screening methods based on artificial or controlled environments with field evaluations of selected individuals is an effective mechanism to minimize the risks associated with these pitfalls.

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